http://www.cjmb.org

Open Access Original Article

Crescent Journal of Medical and Biological Sciences
Vol. 4, No. 3, July 2017, 131–138
eISSN 2148-9696

The Potential Role of Probiotics or/and Prebiotic on Serum Lipid Profile and Insulin Resistance in Alcoholic Fatty Liver Disease: A Double Blind Randomized Clinical Trial

Leila Javadi[1], Mostafa Ghavami[1], Manouchehr Khoshbaten[1], Abdolrasoul Safaiyan[1], Abolfazl Barzegari[1], Bahram Pourghassem Gargari[1*]

Abstract

Objective: Nonalcoholic fatty liver disease (NAFLD) is a chronic disease linked to insulin resistance and fatty acid synthesis. Insulin resistance plays an important role in the pathogenesis of NAFLD causing multiple lipid metabolism disorders. Supplementing probiotics and prebiotics is a complementary therapy in obesity-related disorders, including dyslipidemia and insulin resistance. The objective of this study was to evaluate the effect of probiotic and prebiotic supplementation on serum lipid profile and insulin resistance in patients with NAFLD.

Materials and Methods: In this study, 84 subjects were divided into 4 groups. The first group received probiotic capsules (B.L and L.A: 2*107 CFU/d) and placebo of prebiotic (maltodextrin powder), the second group received prebiotic as sachet (inulin HP: 10 g/d) and placebo of probiotic (fat- and lactose-free milk capsules), the third group received probiotic and prebiotic, and the fourth group received a placebo of probiotic and prebiotic, for 3 months. Anthropometric characteristics, insulin resistance biomarkers and lipid profile were measured for all patients before and after the intervention. The primary outcome of the study was the Homeostasis model of assessment insulin resistance (HOMA-IR) level. The remaining variables (i.e., glucose, insulin, TC, TG, HDL, LDL, weight and BMI changes) were considered represent secondary outcomes.

Results: Results showed that probiotic consumption was able to decrease BMI and weight in all the intervention groups in comparison to the placebo group. The serum levels of HDL and LDL differed significantly in the probiotic and pro- and prebiotic groups in comparison to the placebo group.

In addition, there were no statistically significant reduction in TC, glucose, insulin levels and HOMA-IR in the intervention groups in comparison to the placebo group. Based on the analysis of covariance (ANCOVA) test, there were significant differences in HDL ($P=0.005$) and LDL ($P=0.028$) serum levels between the groups at the end of the study.

Conclusion: Probiotic and prebiotic supplementation may be effective in improving serum lipid profile and insulin resistance markers in patients diagnosed with NAFLD.

Keywords: Probiotic, Prebiotic, lipid profile, insulin resistance, NAFLD

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most significant causes of liver disorders with a prevalence of 20%-30% in modern countries. Its occurrence has enhanced over the past few years primarily due to weight gain, improper lifestyle and eating habit in western societies (1). It is estimated that one-fourth of the American population are overweight, and 80% of them have NAFLD (2).

Due to lack of adequate scientific evidence, the optimized treatment for NAFLD is unknown (3). However, there are various therapeutic procedures based on the adjustment of underlying etiologic effects (4, 5).

Indeed, NAFLD is linked to increased intestinal permeability which is related to the intensity of hepatic steatosis (6). Moreover, intestinal bacterial overgrowth has been stated in 50% of cases (7). In addition, changes in gut microbiota due to stress or improper eating habits has significant effect in the pathogenesis and/or progress of NAFLD (8). Fructooligosaccharides such as inulin, other oligosaccharides, lactulose, resistant starch and dietary fiber enhance response to probiotics.

Probiotics are live microorganisms enhancing host health when administered in large quantities (9). *Bifidobacterium* and *Lactobacillus* are present in several functional foods and nutritional supplements and have significant probiotic properties (10).

Prebiotics such as fiber are slow-growing nutritional compounds regulating gut microbiota and have health interest (11). Prebiotics are able to increase bifidobacteria

Received 30 September 2016, **Accepted** 4 February 2017, **Available online** 25 February 2017

[1]Nutrition Research Center, Department of Biochemistry and Diet Therapy, School of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.
***Corresponding Author:** Bahram Pourghassem Gargari, Tel: +984133362117, Fax: +984133340634, Email: bahrampg@yahoo.com, pourghassemb@tbzmed.ac.ir

or lactobacilli growth and may be useful, in adjusting or declining harmful bacteria growth (12).

Insulin resistance is an important factor in the pathogenesis of NAFLD and is one of the causes of multiple metabolism disorders, which is the result of aggregation of triglyceride in the liver due to increased fatty acids production and high delivery of free fatty acids to the liver (13).

In several clinical trials, useful effects of probiotics on animal and human intestinal microbiota has been proven. Liver fat metabolism can be affected by bacteria and probiotics (14-18). Moreover, few evidence indicate that, probiotics have a protective role in acute liver damage (7) and symbiotic (combination of probiotic and prebiotic) effect liver inflammation and fibrosis in animal models (19). The Food and Drug Administration (FDA) has recognized probiotics as safe (20).

Currently there is no therapeutic or surgical treatment for NAFLD treatment. Various factors have been examined such as thiazolidinedione class of diabetes and vitamin E for NAFLD treatment (14,21-23), however limitations have been reported. In one study, pioglitazone and vitamin E recovered inflammation and hepatic steatosis without recovering fibrosis. Also, pioglitazone significantly increased weight (4.8%) (22,24).

Weight loss through lifestyle modification remains the basis of clinical disease management (25,26). It is indicated that 3%-5% weight reduction, ameliorates biochemical factors and steatosis in NAFLD patients while 10% decrease in body weight is essential for inflammation and NASH recovery (27, 28). In a meta-analysis study including 13 trials, 513 adults with body mass index (BMI) ≥25 kg/m^2 were studied. Symbiotic supplementation resulted in decreased total cholesterol (TC), low-density lipoprotein (LDL) and triglycerides (TG) and increased high-density lipoprotein (HDL) in diabetic trials. Also, symbiotic supplementation decreased fasting insulin and TG. The authors concluded that supplementation with prebiotics and synbiotics is a complementary therapy for diseases related to obesity, such as insulin resistance and dyslipidemia (29).

Considering the limited number of clinical trials regarding the effect of probiotics and prebiotic in NAFLD and the recommendations for accomplishing different studies in the field of NAFLD (30), the current study aimed to assess the effect of probiotics and/or prebiotics alone and in combination on lipid profile and insulin resistance factors in NAFLD patients.

Materials and Methods

Trial Design

A double-blind randomized controlled clinical trial was conducted to evaluate the effect of probiotics (B.L and L.A) and prebiotics (inulin HP), as well as their combined effects, on lipid profile and insulin resistance markers in patients with NAFLD. The primary outcome of the study was HOMA-IR level. The remaining variables (i.e., glucose, insulin, TC, TG, HDL, LDL, weight and BMI changes) were considered represent secondary outcomes.

Participants

A description of the study and written informed consent was provided for all participants at the beginning of study. A questionnaire designed to collect information regarding age, sex, education level and anthropometric indices was completed. Also, lipid profile and insulin resistance tests were administered.

The inclusion criteria were as follows: patients diagnosed with NAFLD willing to participate in the study, men and women aged 20–60 years with serum levels of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) higher than the normal range (reference range for ALT: 0–37 UL/L; reference range for AST: 0–40 UL/L). In addition, NAFLD was diagnosed in patients via ultrasound (Medison SonoAce X6) of the liver and bile ducts, as well as by liver enzymes tests (ALT and AST). The exclusion criteria were as follows: pregnant and lactating women; individuals with cardiovascular, thyroid, kidney, inflammatory, or autoimmune disorders; individuals with diabetes, hepatitis A, B, or C; individuals with hemochromatosis, Wilson disease, or inflammation; using vitamin supplements, including vitamins A, E, and C; and alcohol consumption.

Sample Size

Samples were gathered by convenience sampling method. Participants were divided into 4 groups (probiotics, prebiotics, probiotics and prebiotics, and the placebo group) by random allocation. The required sample size was calculated based on the mean change in HOMA-IR, according to the study of Malaguarnera et al (31). Based on the following formula with an 80% study power and 95% CI, at least 18 patients were allocated in each group. Considering a 20% dropout rate, the sample size was determined to 22 in each group. N= (Z1-α/2+Z1-β)2 (SD12+SD22) /Δ2.

Interventions

Qualified participants were matched for age and sex and randomly divided into 4 groups, including three intervention groups and one control group, using a computer-generated randomization scheme with block sizes of four and eight and an allocation ratio of 1:1:1:1. The first group (n = 21) received probiotics capsules (B.L and L.A: 2*10^7 CFU/d) which were prepared and assessed for their probiotic properties in the Pharmaceutical Nanotechnology, Research Center, Tabriz University of Medical Sciences. The prebiotic placebo, as sachet, was filled with maltodextrin powder which was obtained from the Huirong Trade Company Limited. The second group (n = 21) received prebiotic capsules (inulin HP) purchased from Sensus, Borchwerf, 4704 RG Roosendaal, Netherland and a probiotic placebo, as capsule, was filled with fat and lactose-free milk which was obtained from Nestle S.A; Vevey, Switzerland. The third group (n = 21) received probiotics and prebiotics (B.L and L.A: 2*10^7 CFU/d, plus inulin HP: 10 g/d). The fourth group (n = 21) received

prebiotic and probiotic placebos. The probiotics and the probiotic placebo were administered as 250 mg capsules, and the prebiotics and the prebiotic placebo were administered as 5 g packaged sachets, to be taken twice a day (morning and evening). All treatments were administered for 3 months. To ensure blinding, the allocation was performed by an investigator with no clinical involvement in the study, and the main investigator and statistical data analyst remained blinded until the end of the analysis. Supplements were divided between volunteers in accordance to their allocation code after randomization. We asked all participants to continue taking drugs prescribed by their physicians.

Measurements

Anthropometry Assessment

Patient's height, weight, waist circumference, and hip circumference were measured using standard anthropometric techniques (32). Body weight (with light clothing and without shoes) was recorded to the nearest 0.5 kg by Seca scale (Seca, Hamburg, Germany). Height (without shoes) was measured to the nearest 0.5 cm by Seca stadiometers; BMI and waist to hip ratio (WHR) were measured via the following formulas: [BMI = weight (kg)/ height(m^2)] and [WHR= waist circumference (cm)/hip circumference (cm)].

Blood Tests

Venous blood samples were obtained from participants after 12-hour fasting at the beginning and end of the study. The serum samples were separately centrifuged (Hettich D-78532, Tuttligen, Germany) at 3500 rpm at 4°C and were kept at -70°C until analysis. Afterwards, TC, TG, HDL, LDL, fasting blood sugar (FBS) and insulin were measured by Pars Azmun kits (Tehran, Iran) using auto analyzer machine (Alcyon 300, Abbott. USA). Fasting insulin concentrations were measured by Monobind ELISA. Homeostasis model of assessment insulin resistance (HOMA-IR) was used to determine the degree of insulin resistance using the following formula (14): HOMA-IR = [fasting insulin (mU/L) *fasting blood sugar (mg/dL)]/405.

Preparation of Probiotic

The *Bifidobacterium longum* and *Lactobacillus acidophilus* used in this study were isolated from traditional homemade dairy products. These strains were then screened for the conjugated linoleic acid (CLA) isomerase gene and having cholesterol-lowering function. The CLA-isomerase gene was detected by polymerase chain reaction (PCR), and the cholesterol-lowering effect of the gene product was tested via digestion of cholesterol in culture medium; effective digestion produced transparent medium. The resistance of these bacteria to gastric acid and bile salts was more than 80%, by assessing microbial culture after 3 hours in phosphate-buffered saline for gastric acid and 3–24 hours for bile salts. The selected microbial samples were then cultured to produce the probiotics used in the trial. The capsules were produced by combining 10^7 CFU with fat- and lactose-free milk powder and water until the solution became homogenous. The solution was then lyophilized, and the resulting powder was machine-processed into 250 mg capsules.

Statistical analysis

Data were analyzed using the SPSS 21.0 software. The normal distribution of all variables was confirmed by residual plot. Mean changes of the variables were calculated (end results minus baseline ones). Paired *t* test was used for assessing intra-group changes. Analysis of covariance (ANCOVA) was used for assessing variables mean changes. In the ANCOVA model, mean changes of the variables and groups were considered as dependent variables and fixed factor, respectively. Confounding factors were age, sex, and mean change of BMI and energy intake. Statistical significance was set at a *P* value of <0.05.

Results

Subject Characteristics

In the present study, 88 patients participated. Four subjects were lost before the intervention, thus 84 patients were randomly divided into 4 groups (Figure 1). Afterwards, 9 subjects withdrew because of migration and personal reasons. Participants' mean age and BMI were 42.0 ± 8.9 years and 30.8 ± 4.1 kg/m^2 (23.9-43.2 kg/m^2), respectively. At the baseline, there were no statistically significant differences in demographic parameters as shown in Table 1. Significant differences were observed for anthropometric variables. At the end of the trial, there was significant reduction in BMI and weight in the treatment groups compared to the placebo group.

Lipid profile factors

Comparison of lipid profile in the 4 groups before and after the intervention is presented in Table 2. There were no statistically significant differences in means of serum levels of TC, TG, HDL and LDL among the groups at the baseline. After the intervention, serum levels of TC and HDL in the probiotic, pro- and prebiotic groups and LDL in all the intervention groups changed significantly, with no changes in TG level in comparison to the beginning of the trial. Between-group comparison indicated that HDL ($P = 005$) and LDL ($P = 0.028$) levels changed significantly at the end of study. The serum levels of HDL and LDL changed significantly in the probiotic and Pro- and prebiotic prebiotic groups compared to the placebo group with no differences observed for TG level after the intervention.

Insulin Resistances Parameters

The level of insulin resistance factors in the 4 groups before and after the intervention is presented in Table 3. There were no significant changes in serum levels of glucose, insulin and HOMA-IR among the 4 groups at baseline. In the treatment groups, HOMA-IR decreased significantly at the end of study compared to baseline, but no significant differences were observed for glucose, insulin and

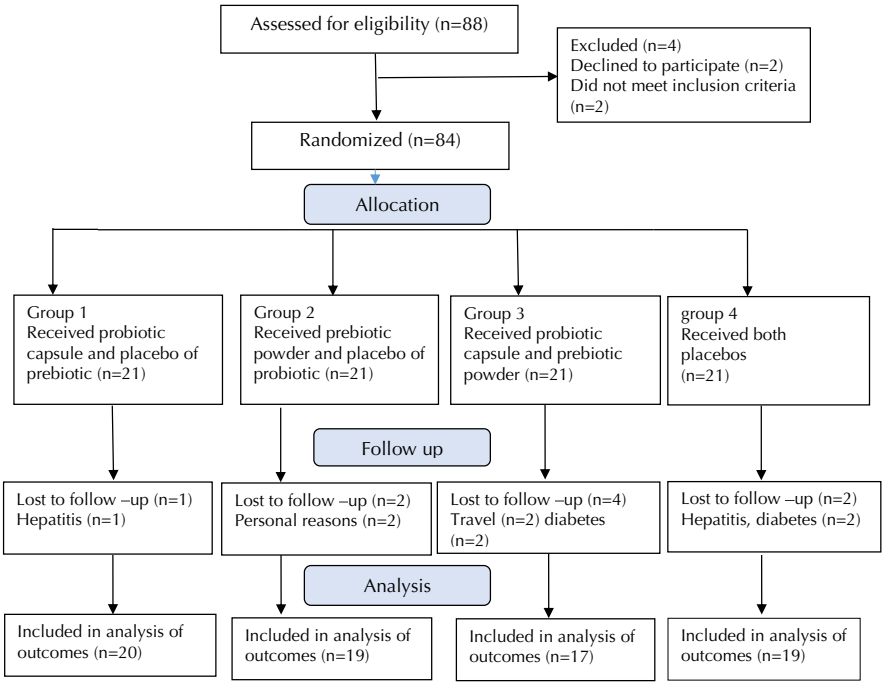

Figure 1. Flowchart of the Study.

HOMA-IR levels between groups at the end of the study.

Discussion

The aim of the study was to appraise the efficacy of probiotics (B.L and L.A) and prebiotics (inulin HP), alone or in combination for serum lipid profile and insulin resistance markers among NAFLD patients.

Our results declares that taking probiotics ($2*10^7$ CFU/d of B.L and L.A) for 3 months significantly reduced weight, BMI and serum levels of LDL and increased HDL level compared to the placebo group, although we did not detect any significant effects on the levels of TC,TG, glucose and insulin among patients with NAFLD. Moreover, TC, HDL, LDL, insulin serum levels and HOMA-IR differed at the end of study in comparison to the baseline levels. Similarly, preclinical studies reported possible probiotic efficiency for weight loss, insulin resistance and hyperlipidemia modulation among animal models (27-30). In addition, 3 RCTs confirmed the effectiveness of probiotics on LDL and HDL serum levels among patients with NAFLD (14,31,33).

Despite our study, several studies declared that probiotics can reduce serum cholesterol without any significant difference in weight and BMI in NAFLD/NASH patients

Table 1. Demographic and Anthropometric Data of the Study Subjects

Variables	Probiotic (n = 20)	Prebiotic (n = 19)	Pro- and Prebiotic (n = 17)	Placebo (n = 19)	*P* Value*
Sex (%)					
Male	17 (85.0%)	16 (84.2%)	14 (82.4%)	13 (68.4%)	
Female	3(15%)	3(15.8%)	3 (17.6%)	6 (31.6%)	
Age (y)	43.90±9.02	38.68±10	43.24±6.95	42.21±9.11	
BMI (kg/m^2)					<0.001
Before	29.91±3.88	30.96±4.39	32.30±4.78	30.38±2.88	
After	29.26±3.59^a	30.38±4.63^a	31.47±4.58^a	30.56±2.88^b	
*P***	0.001	0.005	0.001	0.093	
Weight (kg)					
Before	86.92±12.37	88.45±10.36	89.88±11.92	86.00±11.98	<0.001
After	85.08±12.25^a	86.45±10.54^a	87.91±12.08^a	86.51±12.05^b	
*P***	0.001	<0.001	<0.001	0.072	

Abbreviation: BMI, body mass index. Data are expressed as mean± SD for before and after intervention.
* Resulted from analysis of covariance in the adjusted models sex, age, energy intake, body mass index.
** Resulted from paired sample *t* tests.
Data with different superscript letters are significantly different ($P < 0.05$) to the ANCOVA statistical analysis.

Table 2. Effects of Probiotic, Prebiotic Alone and in Combination on Serum Levels of Lipid Profiles

Variables		Probiotic (n=20)	Prebiotic (n=19)	Pro- and Prebiotic (n=17)	Placebo (n=19)	*P**
TC (mg/dL)	Before	194.20±32.49	189.52±29.06	204.41±32.84	188.94±23.77	0.388
	After	183.45±42.05	183.21±36.45	183.47±40.57	185.47±17.99	0.201
	*P***	0.025	0.284	0.003	0.310	
	*P****	0.457	0.692	0.042		
	MD (95% CI)	-10.75 (-19.98 to -1.51)	-6.31 (-18.31 to 5.68)	-20.94 (-33.89 to -7.98)	-3.47 (-15.20 to 5.09)	
TG (mg/ dL)	Before	165.85±54.17	172.10±73.05	190.64±66.39	150.10±45.68	0.253
	After	152.05±60.41	163.26±66.65	173.35±70.88	149.00±49.86	0.967
	*P***	0.302	0.424	0.161	0.904	
	*P****	0.708	0.949	0.693		
	MD (95% CI)	-12.80 (-38.06 to 12.46)	-8.84 (-31.55 to 13.87)	-17.29 (-42.25 to 7.66)	-1.10 (-20.00 to 17.78)	
HDL (mg/ dL)	Before	42.00±8.30	40.30±1.33	42.33±12.04	38.31±8.01	0.573
	After	47.78±8.37[a]	43.24±10.84[ab]	48.47±7.45[a]	38.63±8.50[b]	0.005
	*P***	0.001	0.250	0.048	0.853	
	*P****	0.003	0.142	0.002		
	MD (95% CI)	5.78 (2.56 to 9.01)	2.94 (-2.25 to 8.14)	6.13 (0.07 to 12.19)	0.32 (-3.22 to 3.85)	
LDL (mg/ dL)	Before	118.54±37.45	115.00±24.86	121.03±27.85	114.55±22.62	0.894
	After	98.76±36.69[a]	102.65±27.87[ab]	101.80±31.43[a]	115.13±19.59[b]	0.028
	*P***	0.001	0.015	0.015	0.912	
	*P****	0.008	0.064	0.011		
	MD (95% CI)	-19.77 (-28.27 to -11.27)	-12.35 (-21.95 to -2.74)	-19.23 (-34.19 to -4.26)	0.58 (-10.29 to 11.45)	

Abbreviations: TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MD, mean difference of within groups (pair sample t test).

Data are expressed as mean ± SD. Data with different superscript letters are significantly different (P<0.05) to the ANCOVA statistical analysis.

* *P* for before of the study resulted from one-way ANOVA test and for after the study resulted from analysis of covariance (ANCOVA) in the adjusted models sex, age, energy intake, body mass index.

** *P* resulted from paired sample *t* tests.

*** *P* resulted from from comparison between each group with placebo group after intervention.

compared with placebo. Also they reported that probiotics can significantly reduce HOMA-IR in NAFLD/NASH patients (14,31,33). One research in the field of mechanisms and metabolism of probiotic action showed that hydrolyzed bile salt can reduce fat concentration and systemic inflammation, plasma leptin, and low-modulate peroxisome proliferator activated receptor-γ (PPAR-γ) in the liver (34-37).

Our study showed that administration of prebiotics (inulin HP: 10 g) for 3 months significantly decreased weight and BMI compared to the placebo group, without any significant effects on TC, TG, LDL, HDL, glucose and insulin levels in NAFLD patients. Nevertheless, LDL serum levels, insulin and HOMA-IR within groups differed significantly.

Similar to our study, Genta et al reported that prebiotics were significantly effective in reducing weight, BMI and HOMA-IR without any change in fasting glucose, TC and HDL in obese women (21). Moreover, insulin level did not change among NASH patients (12), and insulin and glucose levels were constant among obese women (38). In addition, Tovar et al reported that administration of prebiotics was not effective for TC, HDL and glucose in obese women (39).

In spite of our study, several researchers certified that prebiotics can reduce serum cholesterol and LDL in obese adults and obese adults with metabolic syndrome. Also, fasting glucose significantly decreased without any changes in HOMA-IR among women with type 2 diabetes. Reduction in fasting insulin was also observed among obese women and obese adults with metabolic syndrome (21,27,40,41).

The standard dose of prebiotic employed typically in researches is 10% on a weight basis although a variety of doses (5%–20%), and kind of prebiotics (inulin, oligofructose, Synergy1®, lactulose) have been tested. In addition, few studies showed cholesterol-decreasing effect of prebiotics. Various mechanisms have been proven to explain the useful effects of prebiotic fibers on concentration of serum lipids and liver TG animal models, including decreased de novo fatty acid synthesis and SCFA production, reduction in body weight, body fat, inflammation and glycemic control and microbial improvement (42).

The results of our study show that co-administration of probiotics and prebiotics significantly decreased weight, BMI and LDL and significantly increased HDL, without any significant effects on TC, TG, glucose, insulin and HOMA-IR compared with the placebo group among patients with NAFLD. Nevertheless, significant differences were observed for serum levels of weight, BMI, levels of TC, HDL, LDL, glucose, insulin and HOMA-IR within groups.

Similarly, in the study of Malaguarnera et al, there were no significant differences in BMI and serum levels of TG, HDL, LDL, glucose and insulin in patients with non-alcoholic steatohepatitis (31).

Table 3. Effects of Probiotic, Prebiotic Alone and In Combination on Insulin Resistance Factors

Variables		Probiotic (n=20)	Prebiotic (n=19)	Pro-and Prebiotic (n=17)	Placebo (n=19)	*P**
Glucose (mg/dL)	Before	102.55±7.81	100.21±9.62	101.82±5.44	101.10±6.42	0.797
	After	99.77±7.99	98.57±8.93	97.61±5.63	101.63±9.23	0.344
	*P***	0.203	0.528	0.014	0.767	
	*P****	0.375	0.200	0.082		
	MD (95% CI)	-2.77 (-7.18 to 1.63)	-1.63 (-6.96 to 3.69)	-4.20 (-7.44 to -0.96)	0.52 (-3.14 to 4.19)	
Insulin (mu/L)	Before	4.87±1.64	4.56±1.77	5.92±2.58	4.26±1.52	0.062
	After	3.97±2.02	3.47±1.13	4.13±1.62	4.23±1.17	0.344
	*P***	0.007	0.015	0.013	0.909	
	*P****	0.340	0.093	0.164		
	MD (95% CI)	-0.89 (-1.52 to -0.27)	-1.08 (-1.92 to -0.24)	-1.79 (-3.15 to -0.43)	-0.03 (-0.67 to 0.60)	
HOMA-IR	Before	1.23±0.41	1.14±0.49	1.49±0.66	1.06±0.37	0.065
	After	0.98±0.51	0.84±0.29	0.99±0.37	1.06±0.32	0.184
	*P***	0.004	0.016	0.007	0.999	
	*P****	-0.257	0.048	0.075		
	MD(95%CI)	0.25 (-0.41 to -0.09)	-0.29 (-0.53 to -0.06)	-0.50 (-0.84 to -0.15)	-0.00 (-0.16 to 0.16)	

Abbreviation: HOMA-IR, homeostasis model assessment of insulin resistance; MD, mean difference of within groups (pair sample *t* test). Data are expressed as mean ± SD.

* *P* for before of the study resulted from one-way ANOVA test and for after the study resulted from analysis of covariance (ANCOVA) in the adjusted models sex, age, energy intake, body mass index.

** *P* resulted from paired sample *t* tests.

*** *P* resulted from from comparison between each group with placebo group after intervention.

Few studies have investigated the effects of symbiotic therapy on insulin resistance. In constant with the results of few other studies, in this study, significant improvement in HOMA-IR and reduction in FBS and insulin concentrations were observed in patients with NAFLD (23, 31).

These contradictory results are most likely due to the kind of probiotics and prebiotics used, the duration of intervention, the supplement dosage, and the disease studied.

The most important strength of the current study is that it is the first randomized, double-blind clinical trial aimed to study the effect of probiotics and/or prebiotics on serum lipid profile and insulin resistance in acute NAFLD (ALT and AST levels higher than the normal range). In addition, the probiotics used and assessed for their probiotic characteristics were prepared in our laboratory and were screened for the CLA isomerase gene, which has a cholesterol-lowering function.

Our study limitation is that we did not use liver biopsy results to derive a pathology score for disease severity; although liver biopsy is still the gold standard for the diagnosis of NAFLD instead, we chose to use a noninvasive method for disease detection.

In conclusion, in this study, we showed that consumption of probiotics (2*107 CFU/d B.L and L.A) for three months is able to decrease BMI, LDL and increase HDL levels in patients with NAFLD. However, Co-administration of probiotics and prebiotics had positive effects on BMI, TC, LDL and HDL compared to the placebo group at the end of study.

Conflict of Interests

None to be declared.

Ethical Issues

The Ethics Committee of Tabriz University of Medical Sciences agreed the RCT protocol (university ethical code:5/4/7041, 1392/9/2). The study was registered as a clinical trial by the Iranian Registry of Clinical Trials (identifier: IRCT201301223140N6, http://www.irct.ir).

Financial Support

Nutrition Research Centre, Tabriz University of Medical Sciences supported the study financially.

Acknowledgements

The authors would like to acknowledge all the patients who participated in the study. The article is based on the data for PhD by research in nutrition study, which was granted by Nutrition Research Center, Tabriz University of Medical Sciences, Iran (grant No. 5/71/1323, 92/11/1).

References

1. Masarone M, Federico A, Abenavoli L, Loguercio C, Persico M. Non alcoholic fatty liver: epidemiology and natural history. Rev Recent Clin Trials. 2014;9(3):126-33.
2. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Dig Dis. 2010;28(1):155-61. doi: 10.1159/000282080.
3. Zivkovic AM, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. Am J Clin Nutr. 2007;86(2):285-300.
4. Adams L, Angulo P. Treatment of non-alcoholic fatty liver disease. Postgrad Med J. 2006;82(967):315-22.
5. Esposito E, Iacono A, Bianco G, et al. Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. J Nutr. 2009;139(5):905-11. doi: 10.3945/jn.108.101808

6. Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology. 2009;49(6):1877-87. doi: 10.1002/hep.22848.
7. Wigg A, Roberts-Thomson I, Dymock R, McCarthy P, Grose R, Cummins A. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor α in the pathogenesis of non-alcoholic steatohepatitis. Gut. 2001;48(2):206-11.
8. Iacono A, Raso GM, Canani RB, Calignano A, Meli R. Probiotics as an emerging therapeutic strategy to treat NAFLD: focus on molecular and biochemical mechanisms. J Nutr Biochem. 2011;22(8):699-711. doi: 10.1016/j.jnutbio.2010.10.002.
9. Joint FAO/WHO Working Group. Guidelines for the evaluation of probiotics in food: report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. London, Ontario, Canada: 2002.
10. Plaza-Diaz J, Gomez-Llorente C, Abadia-Molina F, et al. Effects of *Lactobacillus paracasei* CNCM I-4034, Bifidobacterium breve CNCM I-4035 and *Lactobacillus rhamnosus* CNCM I-4036 on hepatic steatosis in Zucker rats. PLoS One. 2014;9(5):e98401. doi: 10.1371/journal.pone.0098401.
11. Pineiro M, Asp N-G, Reid G, et al. FAO Technical meeting on prebiotics. J Clin Gastroenterol. 2008;42:S156-S9. doi: 10.1097/MCG.0b013e31817f184e.
12. Daubioul C, Horsmans Y, Lambert P, Danse E, Delzenne NM. Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. Eur J Clin Nutr. 2005;59(5):723-6. doi: 10.1038/sj.ejcn.1602127.
13. Medina J, Fernández-Salazar LI, García-Buey L, Moreno-Otero R. Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis. Diabetes Care. 2004;27(8):2057-66.
14. Aller R, De Luis D, Izaola O, et al. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci. 2011;15(9):1090-5.
15. Loguercio C, Federico A, Tuccillo C, et al. Beneficial effects of a probiotic VSL# 3 on parameters of liver dysfunction in chronic liver diseases. J Clin Gastroenterol. 2005;39(6):540-3.
16. Ma X, Hua J, Li Z. Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. J Hepatol. 2008;49(5):821-30. doi: 10.1016/j.jhep.2008.05.025.
17. Osman N, Adawi D, Ahrné S, Jeppsson B, Molin G. Endotoxin-and D-galactosamine-induced liver injury improved by the administration of Lactobacillus, Bifidobacterium and blueberry. Dig Liver Dis. 2007;39(9):849-56.
18. Xu RY, Wan YP, Fang QY, Lu W, Cai W. Supplementation with probiotics modifies gut flora and attenuates liver fat accumulation in rat nonalcoholic fatty liver disease model. J Clin Biochem Nutr. 2011;50(1):72-7. doi: 10.3164/jcbn.11-38.
19. D'Argenio G, Cariello R, Tuccillo C, Mazzone G, Federico A, Funaro A, et al. Symbiotic formulation in experimentally induced liver fibrosis in rats: intestinal microbiota as a key point to treat liver damage? Liver Int. 2013;33(5):687-97. doi: 10.1111/liv.12117.
20. Gratz SW, Mykkanen H, ElNezami HS. Probiotics and gut health: a special focus on liver diseases. World J Gastroenterol. 2010;28;16(4):403-10.
21. Genta S, Cabrera W, Habib N, et al. Yacon syrup: beneficial effects on obesity and insulin resistance in humans. Clin Nut. 2009;28(2):182-7. doi: 10.1016/j.clnu.2009.01.013.
22. De los Reyes-Gavilan CG, Delzenne NM, González STI, Gueimonde M, Salazar Garzo N. Development of functional foods to fight against obesity: opportunities for probiotics and prebiotics. Agro Food Industry Hi-Tech. 2014;25:35-9.
23. Eslamparast T, Poustchi H, Zamani F, Sharafkhah M, Malekzadeh R, Hekmatdoost A. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. Am J Clin Nutr. 2014;99(3):535-42. doi: 10.3945/ajcn.113.068890.
24. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. A natural solution for obesity: bioactives for the prevention and treatment of weight gain. A review. Nutr Neurosci. 2015;18(2):49-65. doi: 10.1179/1476830513Y.0000000099.
25. Peters HP, Boers HM, Haddeman E, Melnikov SM, Qvyjt F. No effect of added β-glucan or of fructooligosaccharide on appetite or energy intake. Am J Clin Nutr. 2009;89(1):58-63. doi: 10.3945/ajcn.2008.26701.
26. Verhoef SP, Meyer D, Westerterp KR. Effects of oligofructose on appetite profile, glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. Br J Nutr. 2011;106(11):1757-62. doi: 10.1017/S0007114511002194.
27. Pourghassem Gargari B, Dehghan P, Aliasgharzadeh A, Asghari Jafar-abadi M. Effects of high performance inulin supplementation on glycemic control and antioxidant status in women with type 2 diabetes. Diabetes Metab J. 2013;37(2):140-8. doi: 10.4093/dmj.2013.37.2.140
28. Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58(8):1091-103. doi: 10.1136/gut.2008.165886.
29. Beserra BT, Fernandes R, do Rosario VA, Mocellin MC, Kuntz MG, Trindade EB. A systematic review and meta-analysis of the prebiotics and synbiotics effects on glycaemia, insulin concentrations and lipid parameters in adult patients with overweight or obesity. Clin Nutr. 2015;34(5):845-58. doi: 10.1016/j.clnu.2014.10.004
30. Tarantino G, Finelli C. Systematic review on intervention with prebiotics/probiotics in patients with obesity-related nonalcoholic fatty liver disease. Future Microbiol. 2015;10(5):889-902. doi: 10.2217/fmb.15.13.
31. Malaguarnera M, Vacante M, Antic T, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Dig Dis Sci. 2012;57(2):545-53. doi: 10.1007/s10620-011-1887-4.
32. Lohman T, Roache A, Martorell R. Anthropometric standardization reference manual. Medicine & Science in Sports & Exercise. 1992;24(8):952.
33. Wong V, Won G, Chim A, et al. Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. Ann Hepatol. 2013;12(2):256-62.
34. Peterson C, Sharma V, Elmén L, Peterson S. Immune homeostasis, dysbiosis and therapeutic modulation of the gut microbiota. Clin Exp Immunol. 2015;179(3):363-77. doi: 10.1111/cei.12474.
35. Stenman L, Waget A, Garret C, Klopp P, Burcelin R, Lahtinen S. Potential probiotic Bifidobacterium animalis

ssp. lactis 420 prevents weight gain and glucose intolerance in diet-induced obese mice. Benef Microbes. 2014;5(4):437-45. doi: 10.3920/BM2014.0014.
36. Savcheniuk O, Kobyliak N, Kondro M, Virchenko O, Falalyeyeva T, Beregova T. Short-term periodic consumption of multiprobiotic from childhood improves insulin sensitivity, prevents development of non-alcoholic fatty liver disease and adiposity in adult rats with glutamate-induced obesity. BMC Complement Altern Med. 2014;14:247. doi: 10.1186/1472-6882-14-247.
37. Kang J-H, Yun S-I, Park M-H, Park J-H, Jeong S-Y, Park H-O. Anti-obesity effect of Lactobacillus gasseri BNR17 in high-sucrose diet-induced obese mice. PLoS One. 2013;8(1):e54617. doi: 10.1371/journal.pone.0054617.
38. Dewulf EM, Cani PD, Claus SP, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. Gut. 2013;62(8):1112-21. doi: 10.1136/gutjnl-2012-303304.
39. Tovar AR, del Carmen Caamaño M, Garcia-Padilla S, García OP, Duarte MA, Rosado JL. The inclusion of a partial meal replacement with or without inulin to a calorie restricted diet contributes to reach recommended intakes of micronutrients and decrease plasma triglycerides: A randomized clinical trial in obese Mexican women. Nutr J. 2012;11:44. doi: 10.1186/1475-2891-11-44.
40. de Luis Román DA, De la Fuente B, Jáuregui OI, et al. Double blind randomized clinical trial controlled by placebo with an alpha linoleic acid and prebiotic enriched cookie on risk cardiovascular factor in obese patients. Nutr Hosp. 2011;26(4):827-33. doi: 10.1590/S0212-16112011000400024.
41. Dehghan P, Gargari BP, Jafar-Abadi MA, Aliasgharzadeh A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. Int J Food Sci Nutr. 2014;65(1):117-23. doi: 10.3109/09637486.2013.836738.
42. Parnell JA, Raman M, Rioux KP, Reimer RA. The potential role of prebiotic fibre for treatment and management of non-alcoholic fatty liver disease and associated obesity and insulin resistance. Liver Int. 2012;32(5):701-11.